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Resistance to the two-spotted spider mite,

Tetranychus urticae (Koch), in New Guinea Impatiens

by

Sabri Hassan Al-Abbasi

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TABLE OF CONTENTS

INTRODUCTION

Since World War II, spider mites have been considered serious phytophagous pests for distinct food, fiber, and ornamental plants. They may cause very heavy crop losses through damaging leaf surfaces, the stomata, and spongy parenchyma, and they may inject toxic materials into the leaf and interfere with vital processes (Huffaker et al., 1969). Up until recently, it was possible to control most species of spider mites by use of acaricides. These include the organophosphorous compounds, organochlorines, nitrophenols, quinoxalines, and the formamidines (Herne et al., 1979). Recently, however, many populations of mites have developed marked resistance to such acaricides. Therefore, it has been necessary either to keep developing new compounds that mites have no resistance to, or breed new plant varieties that are resistant to the pest. The latter has been considered by many growers to have great potential for pest control, and it is compatible with other methods of integrated control (Dahms, 1972).

The two-spotted spider mite, *Tetranychus urticae* (Koch), is one of the most important spider mites in the temperate regions of the world (Kono and Papp, 1977), with long range of host plants. It is a major pest on casava, castorbean, chrysanthemum, cotton, cucumber, geranium, grape, hop, potato, soybean, strawberry, sugarbeet, tomato, and a great many other plants.

Breeding plants resistant to this pest has been successfully achieved in such plants (Ponti, 1977). Little work, however, has been done with other ornamentals.

The biggest problem with growing New Guinea Impatiens plants is their susceptibility to the two-spotted spider mite. The problem is particularly severe in the greenhouse. While working with these plants at the Iowa State University Horticulture greenhouse, variation in susceptibility to the pest among different *Impatiens* genotypes has been observed. Therefore, a concern of more detailed study of the resistance to the mite has been established.

The objectives of this work are to evaluate some of the Impatiens genotypes concerning their resistance to the two-spotted spider mite and to investigate physiological and morphological factors that may be related to the resistance mechanism.

LITERATURE REVIEW

The two-spotted spider mite has been known under many names: the glasshouse spider mite, red spider mite, and red spider (Jeppson et al., 1975). These common names included *Tetranychus cinnabarinus* (Boisduval), *Tetranychus telarius* (Linnaeus), *Tetranychus bimaculatus* (Harvey), *Tetranychus urticae* (Koch), and up to 59 synonyms (Jeppson et al., 1975; Kono and Papp, 1977; Smith and Baker, 1968) now considered as different species. According to the work of Boudreaux and Dosse (1963), *Tetranychus urticae* (Koch) is the valid name for the two-spotted spider mite.

The two-spotted spider mite has been studied by a large number of investigators. Literature reviewed here is concerned with some of the biological and ecological studies and the host plant relationship.

Biology and Ecology

The two-spotted spider mite, as cited by Nelson and Stafford (1972), was found to have the haploid number of chromosomes (n=8) in the male and the diploid number of chromosomes (2n=16) in the female. The stages in the life cycle are egg, larva, protonymph, deutonymph, and adult (Van de Vrie et al., 1972). Each active immature stage is followed by a quiescent state (Huffaker et al., 1969). These stages and state may vary in their susceptibility to environmental stresses. Cagle (1949) found the average life period was 28 days for adult males and 22 days for adult females. Harrison and Smith (1961) found that mean incubation periods for the eggs ranged from 2.4 days at 32.5°C to 33.2 days at 11.5°C. Hussey et al. (1957) found that the number of generations of the two-spotted spider mite and their seasonal activity are correlated with temperature as modified by other factors, such as the nutritional conditions of the host plant. In greenhouses, they found that the duration of egg stage for females decreased from 5.9 days at 21.1°C to 2.3 days at 35°C, and that the duration of the larva stage decreased from 7.4 days at 21.1°C to 3.4 days at 32.2°C but rose to 4.1 days at 36.7°C. As with other spider mites, the maturing males of this species locate and remain near the quiescent deutonymphs with mating taking place immediately after cedysis of the young females (Van de Vrie et al., 1972).

Humidity also is important in spider mite ecology. Winston (1963) stated that both gain from and loss to the atmosphere of water by mites is fundamental to their existence. The amount of feeding they do and thus the injury they cause may be related to their water balance (Wharton, 1963). Boudreaux (1958), in a study of four species of spider mites, found a reduced egg production and oviposition period and increased mortality of newly hatched larvae under high humidity and postulated that water loss by evaporation determines the amount that the mite can take in by feeding. Thus, under low humidity, both intake of nutrients and reproduction will be greater.

Effects of light on spider mites also have been studied. The reaction to light of two-spotted spider mites was studied by Suski and Naegele (1963). They reported that this species shows sedentary and dispersal phases. On a fresh leaf of favorable food, the mite showed little or no response to light, but as soon as the leaves are destroyed by mite feeding, a food deficiency and desiccation occurs causing an increase in activity in the mite population which interacts with some still unknown factors leading to a taxis-type light response for new food resources. McEnroe and Dronka (1966, 1969) studied the reaction of adult females of the two-spotted spider mite to the light of various wavelengths. They reported that the color vision of the two-spotted spider mites is shown to be the near-ultraviolet and green region. Another activity of spider mites found to be affected by light is webbing. Gerson (1979), using different light conditions, found that the largest amount of silk was spun under continuous light conditions, less under an alternating light regime of 14 hours light and 10 hours darkness, and least in total darkness.

Locomotion, spinning, and feeding, important activities in spider mite biology, were studied by many investigators. Saito (1977) found a very close correlation between the walking activity of the females of the two-spotted spider mites and their spinning. Hazan et al. (1975) found that feeding, at its best conditions of 24°C and 38% relative humidity, best promotes spinning. Starved mites, however, web a large amount of silk before they die (Hazan et al., 1974), indicating that feeding is not necessary for spinning, and that materials required for this silk are carried over from the preadult stages.

Hussey and Parr (1963) reported that the two-spotted spider mites spread by three different methods: migration of the teneral females to oviposition sites, migration from heavily infested plants by dropping off, and migration over the soil surface in accordance with the plane of polarized light. The effect of these dispersal mechanisms is to restrict mite population to one portion of a greenhouse from season to season, with relatively slow lateral spread from these centers during each growing season (Van de Vrie et al., 1972).

There are many ways that a host plant might affect a spider mite population. The structure of the leaf surface, thickness of the cuticle, chemical composition of the saps, osmotic pressure within a cell, microclimate in the canopy, and most probably many others (Jesiotr et al., 1979). Of the above factors, numerous workers, such as Henneberry (1962), Rodriguez (1951), and Suski et al., (1975), investigated the effect of the application on plants of the mineral fertilizers on the mite populations. They reported contradictory results. None of the other physical or the physiological factors have been thoroughly investigated at present.

Host-Plant Resistance to Spider Mite

Painter (1951) defined resistance of a plant to an insect pest as "the relative amount of heritable qualities possessed by the plant which influence the ultimate degree of damage done by the insect." The resistance to pests can be caused by one or more of several different kinds of mechanisms. Painter (1951) divided them into three main mechanisms. These are: (1) nonpreference, which is shown by plants that are unattractive or unsuitable for food, shelter, or oviposition by an insect; (2) antibiosis, which adversely affects insect mortality, size, and life history (Owens, 1975); (3) tolerance, which enables a host plant to withstand an attack by insects without suffering severe damage; and (4) pest avoidance, which is expressed as a tendency to escape infestation (Russell, 1978).

Nonpreference

Any inherited characteristic of a host plant that discourages the feeding, colonization or oviposition of an animal pest makes it "nonprefered" by that pest (Russell, 1978). Ponti (1977) used the term "nonacceptance" instead of the term "nonpreference". He indicated that during the orientation phase of an insect, a plant is rejected as a host regardless of whether it is offered singly or together with other plants. However, the term "nonpreference" has been generally accepted and used to mean nonacceptance by many breeders.

Nonpreference may be attributed to morphological, physiological or biochemical factors in the host plants (Russell, 1978). Beck (1965), Owens (1975), Painter (1951), and Russell (1978) exhibited many examples regarding this subject.

Antibiosis

The rate of population increase on a host plant is reduced by antibiosis because it causes the death of the pest, or decreases its rate of development or reproductive potential (Russell, 1978). This was attributed either to the presence of toxic secondary compounds (Fraenkel, 1959) and/or lack of essential nutrients (Thorsteinson, 1960).

Tolerance

Tolerance, as stated by Russell (1978), does not in any way restrict or hinder the colonization of a host plant by a pest nor does it affect the development or reproduction of that pest. It does, however, reduce the damage to the host plant that is caused by a pest, since a tolerant variety will grow more normally and produce higher yields than an intolerant variety when they are both infested to the same extent by a pest.

Tolerance, as stated by Owens (1975), can be divided into at least two categories, endurance and repair. Endurance is often encountered in insects with piercing-sucking mouth parts, whereas repair tolerance is associated with insects with biting mouth parts.

Beck (1965) dropped tolerance from the resistance considerations because of its implication to a biological relationship between insects and plants which is quite different from resistance in the strict sense. He defined resistance as being "the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the probability of successful utilization of that plant as a host by an insect species, race, biotype, or individual."

Pest avoidance

Some plants avoid infestations because they are not at a very susceptible stage when pest populations are at their peak (Russell, 1978). In some apple cultivars, as reported by Briggs and Alston (1969), the infestation by different species of insect pest does not occur because the buds of the plants do not break until after the main hatching or emergence period of the pest. Many breeders referred to this type of resistance as pseudoresistance.

Although it is not always necessary to identify the bases of resistance present to develop an insect resistant line, it is essential to distinguish between tolerance, nonpreference, and antibiosis (Owens, 1975). The terminology involving the resistance mechanisms of plants to insects is applied to resistance studies of plants to spider mites, since there are no essential differences in resistance to plant parasites (Ponti, 1977). Even though spider mites are not insects, they have been treated, concerning their relationship to host plants, as insects and most of the plant-spider mite studies are published in the same publications as those studies concerning insect resistance.

Resistance to spider mites, with different levels, has been found in many different crops, including a few ornamentals. The following is an exploration through some studies concerning plant resistance to the two-spotted spider mite, Tetranychus urticae (Koch) and its synonyms.

In chrysanthemum plants, the reproduction rate and life span of the two-spotted spider mite were investigated by Markkula et al. (1969), who found differences in abundance of spider mite on different species of chrysanthemum. They also reported that potassium deficiency increased mite reproduction on some of the cultivars and decreased it on others.

Resistance to the two-spotted spider mite was demonstrated by screening seedlings of some cotton plants in the greenhouse (Schuster et al., 1972; Schuster and Maxwell, 1976). Also, Schuster et al. (1972) found resistance in some races in the field when they infested plants with laboratory-reared two-spotted spider mites. Bailey et al. (1978) studied 16 cotton genotypes with different morphological characters. They stated that selection of resistant cotton genotypes based on morphological traits may be explored in future host resistance studies.

In cucumber, the presence of cucurbitacin has been reported to be associated with a toxic effect of cucumber leaves to the two-spotted spider mites (DaCosta and Jones, 1971; Kooistra, 1971). DaCosta and Jones (1971) believed that cucurbitacins evolved in wild cucumbers as a mechanism to protect them from this pest. Soans et al. (1973) found a bitter cucumber variety that was as susceptible to the two-spotted spider mite as a nonbitter one. They concluded that bitterness was not always associated with resistance to mites. Ponti (1979) found similar examples of bitter susceptible plants. He explained the results as a close linkage between genes for resistance and bitterness rather than both characteristics being controlled by a single gene.

A collection of eggplants and related _Solanum_ species from different parts of the world were tested by Schalk et al. (1975) for their resistance to the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval). Tolerance to mite feeding damage was found in some species, antibiosis was found in others, and nonpreference for feeding and oviposition was found for others. Some of these species showed antibiosis and nonpreference simultaneously. Immunity was found in some accessions. Glandular hairs on these plants were found to trap the mites on the leaf surface. Also, the exudate from these hairs may have had repellent, deterrent, or lack of arrestant properties.

Similar observations on mite fecundity were noted by Soans et al. (1973) on some of the same cultivars which were used by Schalk et al. (1975) when they were studying the resistance of eggplants to the two-spotted spider mite.

In regard to the resistance to the two-spotted spider mite by geranium plants, Snetsinger et al. (1966) reported that susceptible geranium plants are easy to detect by their yellow leaves and whitish streaks caused by feeding injury of the pest. They also stated that resistance and susceptibility can be measured objectively by rearing spider mites on detached geranium leaves and recording the number of eggs laid per female. They did not report the characteristics that promote the resistance, but they suggested that certain types of secretory hairs on both surfaces of the leaves may be involved.

In hop plants, attention was paid to studying the chemical nature of resistance. Regev and Cone (1975) submitted evidence that farnesol serves as a component of the two-spotted spider mite's male sex attractant-arrestant system. Since mites are not known to synthesize farnesol, Regev and Cone (1975) designed a study to investigate whether the hop varieties differ in farnesol content and to test the association of the mite populations with farnesol content of different hop varieties. They found a positive correlation between farnesol content of the plants and susceptibility to two-spotted spider mite infestation. In bioassay study, Regev and Cone (1980) isolated and identified another male attractant chemical, citronellol, from pharate females. In their work, they found that males were highly attracted to 10 ppm synthetic citronellol.

The inheritance of resistance to the two-spotted spider mite in strawberry plants was studied by Chaplin et al. (1968). They indicated that it was partially dominant and controlled by multiple genes, and that selfing resulted in a loss of resistance. It was speculated that this might be due to nonadditive gene action and/or a loss of vigor. They also indicated that breeding for mite resistance in strawberry is feasible. Later, Chaplin et al. (1970) studied the breeding behavior of mite-resistant strawberry plants by means of backcrosses, sib crosses, outcrosses, and combinations of selfed lines. They found that backcrosses to the resistant parents resulted in intermediate progeny with a resistance level nearer to that of the less resistant parent. Backcrosses to the susceptible parent also resulted in intermediate progeny reacting more like the resistant parent. Outcrosses gave progenies similar to those of backcrosses but with less spread in resistance. Progeny of crosses between certain resistant, selfed selections showed alsmot complete dominance for mite resistance.

Sances et al. (1979) studied morphological responses of strawberry leaves to infestations of two-spotted spider mite. They reported that stomatal closure was an important host-plant response associated with spider mite infestation on these plants. They attributed the loss in productivity of infested strawberry plants to the stomatal closure which reduces the CO_2 uptake, thus, lowering the photosynthesis rate. In this study, they also found significant differences in stomatal opening between mite-infested and mite-free leaflets.

The two-spotted spider mite was reported by Patterson et al. (1974) as the limiting factor in growing susceptible tobacco cultivars in the greenhouse. In their study, they rated the plants due to the injury caused by infestation and stated that resistance associated with a viscid secretion from trichames took 2 forms: toxicity and entrapment, either one resulting in mite death.

In tomato, the potential for controlling the spider mites by host-plant resistance has been demonstrated by Gilbert et al. (1966) and Stoner and Stringfellow (1967). They showed that tomato varieties differ significantly in resistance to carmine spider mite. Stoner et al. (1968) and Stoner (1970) found a positive correlation between the level of resistance in tomato plant and the density of glandular hairs on their leaves, confirming the suggestion of McKinny (1938) and Johnson (1956).

Patterson et al. (1975), concerning the chemical nature of tomato resistance to the two-spotted spider mite, reported two compounds present in leaves of resistant varieties which were the most toxic and repellent to the two-spotted spider mites. These compounds were sesquiterpenoids.

Finally, no information has been found in the literature concerning the study of resistance to the two-spotted spider mite or any other mite in New Guinea *Impatiens* plants.

MATERIALS AND METHODS

Initial Screening and Evaluation of Plants

Twenty-seven lines of New Guinea *Impatiens* plants were cloned in the spring and again in the fall of 1979 (Table 1). After rooting, they were planted in 7.6 cm pots and allowed to grow for 4 weeks. Three replications of each line were randomly assigned in 1.5 x 1.5 x 1.2 m plastic cages to isolate them during screening from other plants in the greenhouse.

When inoculated, the host plants were 15-20 cm in height and had 14-16 leaves. Each plant was inoculated with 10-12 adult females of two-spotted spider mites which had been reared and maintained in the cages on susceptible *Impatiens* lines. The susceptible plants were replaced frequently to maintain a vigorous mite population. The mites were transferred from plant to plant with a small brush. The pot rims were rubbed with vaseline to restrict the migration of mites from plant to plant in the cages. Direct contact among host plants was eliminated for the same purpose.

At weekly intervals for eight weeks, differences in the degree of damage caused to plants by mites were judged and plants were scored on a scale of 1 to 5 (1 = highly resistant, no leaf yellowing; 2 = resistant, leaf yellowing indicated; 3 = intermediate, half of the leaf yellowed and

Table 1. *Impatiens* lines used in the screening procedure for resistance to the two-spotted spider mite, *Tetranychus urticae* Koch, their pedigree, and parentage ratio

Line	Pedigree	Parentage ratio
7	P.I.354251[a]	1/1
12	P.I.354256	1/1
14	P.I.354258	1/1
16	P.I.354260	1/1
21	P.I. 354265	1/1
October Charm	Not available	-
Hot Pants	Not available	-
Stoplight	Not available	-
452-1[b]	P.I.354252	1/2
	P.I.354254	1/2
473-1[b]	P.I.354259	1/2
	452-1	1/2
474-2[b]	452-1	1/4
	P.I.354259	1/4
	P.I.349588	1/4
	P.I.354265	1/4
622-1[b]	P.I.354253	1/4
	21	1/4
	12	1/4
	P.I.349588	1/8
	P.I.354252	1/8
625-1[b]	P.I.354255	1/8
	P.I.354259	3/8
	P.I.354252	1/4
	P.I.354257	1/4
7884-C[b]	P.I.354254	1/8
	P.I.354252	3/8
	625-1	1/2
7729-8[b] and	P.I.354253	1/4
7729-9[b]	P.I.354252	5/16
	P.I.349588	1/4
	21	1/8
	P.I.354257	1/16

[a]P.I. indicates a plant introduction number.

[b]Iowa State University selection.

Table 1. (Continued)

Line	Pedigree	Parentage ratio
77109-1D[b]	P.I.349588	1/4
	21	1/4
	460-2 (unknown parents)	1/2
Ring Master	12	7/16
	P.I.349588	1/4
	P.I.354252	3/16
	P.I.354253	1/16
	P.I.354254	1/16
78236[b]	Ring Master	1/2
	Unknown	1/2
Pink Satin	P.I.354252	1/2
	Tangerine[c]	1/2
Purple Silk	16	1/2
	Tangerine	1/2
Rainbow Star and	P.I.354264	1/2
Star Fire	Tangerine	1/2
Summer Star	P.I.354262	1/2
	P.I.349629	1/2
Red Coat	P.I.354262	1/4
	P.I.349588	1/4
	P.I.354254	1/4
	P.I.354259	1/4
Chariot	P.I.354253	1/8
	P.I.354254	1/8
	P.I.354258	1/8
	P.I.354255	1/8
	P.I.354252	1/4
	P.I.354257	1/4
Red Magic	Not available	

[c]Commercial line.

necrotic; 4 = susceptible, leaf entirely or almost entirely yellowed and necrotic with web; and 5 = highly susceptible, leaf dried or died). The score for each line in both spring and fall was obtained by averaging the replicate scores at the end of the eighth week each time the experiment was conducted. The results were statistically analyzed.

Determination of Leaf Cuticle

Leaf cuticle of the same lines was isolated and weighed using the method of Holloway and Baker (1968). Fifty disks (1.6 cm in diameter each) from each line were soaked for 4 hours in the zinc chloride-hydrochloric acid solution to remove the cuticle. A correlation test of the average amount of cuticle of 3 replications per line with the line scores was done.

Crosses and Selfs Among Plants

Sixteen lines with a wide range of resistance levels were selected from the original 27 that were tested, crossed reciprocally, and selfed during September, October, and November of 1979. Seeds from successful reciprocal matings were sown in December and seedlings were transplanted in 7.6 cm pots when they reached a height of 3 to 5 cm. Plants were allowed to grow to blooming stage. Hybrids were divided into 2 groups on the basis of having similar flower and leaf shape. Only the healthiest plant of each group was kept and cloned. All progeny of selfed plants were cloned.

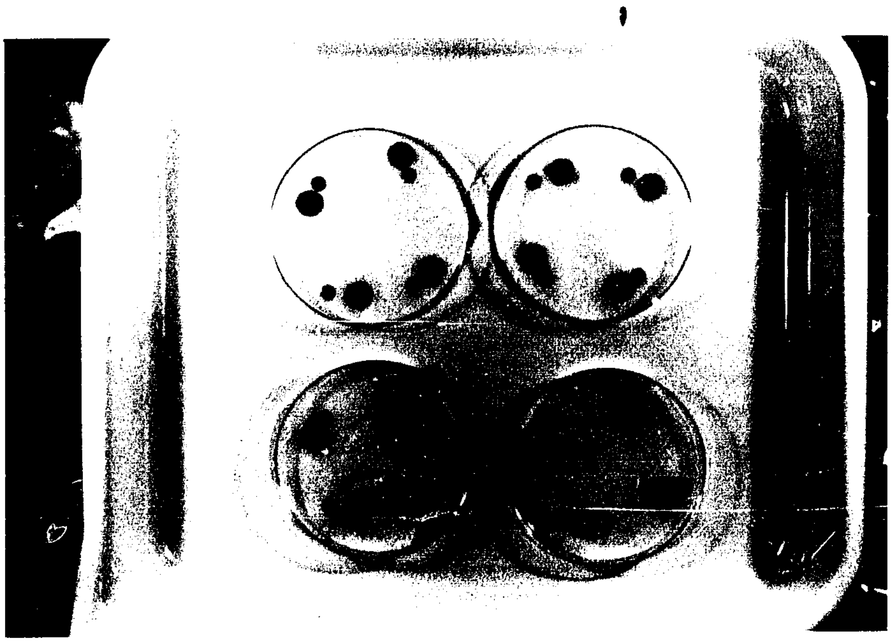
Progeny evaluation

Feeding preference

For hybrids   Feeding preference tests were run among the 4 selected hybrids in each set of reciprocal crosses. Leaf disks (1.6 cm in diameter) were cut from leaves of each of these plants and one dish from each plant was put upside down close to the edge of a thick filter paper in a 10-cm plastic petri dish. The filter paper was underlain with 4 layers of moistened cheesecloth. Twenty adult female mites that had been starved for 6 hours were put in the center of the petri dish (Figure 1). This procedure was replicated 4 times. After applying vaseline on their top edges, the uncovered petri dishes were put in a growth chamber at $25^{o}C \pm 2^{o}C$ with continuous illumination. After 12 hours, the number of mites on each disk was counted under the microscope and the average number of mites per disk per set of reciprocal crosses was calculated. Comparisons by T-test among the sets were done.

For selfed progenies   The same procedure was used in testing the progeny of the selfed plants except the petri dishes were replaced by plastic trays to make enough room for the disks of the higher number of individuals. The total number of mites on disks of each individual was counted and analysis of variance was done.

Figure 1. Four replicates with leaf disks used in the food preference test for each of the successful reciprocal hybrids



Damage evaluation Only the hybrid which showed the lowest feeding preference for mites from each set of reciprocal crosses and 3 randomly selected plants from each selfing progeny were used. The hybrids were replicated 3 times, and the 3 selected plants from each selfing progeny were considered as 3 replicates. The same rating procedure as that used with the parents was run with progeny except it was only run once. The mean scores of the replicates at the end of the eighth week were statistically analyzed.

Tests on Selected Parent and Progeny Plants

Clones of the lines '452-1', '474-2', '7729-8', '7729-9', '77109-1D', 'Hot Pants', 'Ring Master', '7729-9x7729-8', '7729-9x77109-1D', '7729-9xRing Master', '7729-9xHot Pants', '7729-9x452-1', '77109-1DxHot Pants', '77109-1Dx452-1', '452-1x474-2', '7729-8x77109-1D', and the most nonpreferred plant from the selfed progeny of the line '7729-9', were selected for further tests. All others were discarded.

Oviposition response tests

Leaf disk method: Based on the procedure suggested by Ponti and Inggamer (1976), three disks (1.6 cm in diameter) from mature leaves per selected clone were cut and randomly placed upside down on the filter paper. Young adult female mites were selected from a population reared on susceptible

Impatiens plants in the greenhouse and were placed on the disks with a rate of 1 mite per disk. Three replications were made. The trays were put in a growth chamber at 25°C during 14 light hours and 17°C during 10 dark hours for 120 hours (5 days). The eggs deposited by mites were counted under the microscope on each disk. The average number of eggs per disk per adult female was calculated and statistically tested.

Rings method: Three rings of Tanglefoot, each about 2 cm in diameter, were made on the upper surface of 3 randomly selected leaves on each of the 17 lines. One young adult female mite was put in each ring. Plants were placed randomly in a growth chamber with conditions to that in the leaf disks method. After 5 days, eggs were counted under the microscope. The average number of eggs per female per ring was calculated for each line and the data were statistically analyzed.

Life cycle tests

The rings method After counting the eggs deposited in the rings method of oviposition response test, all eggs and nymphs were removed from the rings. The females were left for a while and then removed along with all but 3 new eggs. After 10 days and on each following day, the rings on the leaves were checked for the first egg laid by any newly hatched female. Once an egg was found, the time was recorded. This test ended when an egg had been found laid in every ring of all plants. The life cycle of the mites on each line was calculated by averaging the time taken to get the first new egg in each of the 3 replications. The results were statistically analyzed.

The whole leaf method: New plants of the 17 lines were used. Lanolin was applied around the petioles of 3 randomly selected leaves on each plant. Three adult female mites were taken from each previously used clonal plant and put on the leaves of a plant of the same clone at rate of 1 mite per leaf. Plants were randomly assigned in the growth chamber with the same conditions as used in the previous experiment. After 3 days, the leaves were checked for oviposition and the mites were removed with all but 3 new eggs. The remaining steps of this procedure are similar to that in the previous test.

Food preference test

Detached leaves, 1 from each line, were randomly arranged near the edge of a circle of polyethylene laid on 8 moistened cheesecloth layers in a plastic tray (Figure 2). The petiole of each leaf penetrated the cheesecloth and the blade laid on the polyethylene. Fifty-one, six-hour-starved adult female mites were put in the center of the circle. With 3 replications, trays were put in a growth chamber for 12 hours at

Figure 2. A replicate with whole leaves used in the food preference test for the selected *Impatiens* parents and progenies

$25^{\circ}C$ with continuous illumination. The mites on each leaf were counted and the average number of mites per leaf per line was calculated and statistically analyzed.

pH preference test

Using the procedure of LaMotte et al. (1973), the pH of leaf filtrates of the lines was measured using a microelectrode. Each measurement was repeated 3 times. The average was calculated and statistically tested. Correlation coefficient was calculated between the pH of leaf filtrates and feeding preference.

Size of eggs, adult females, and adult males

The mites on the plants used in oviposition response test (rings method) and of the life cycle test (rings method) were allowed to infest the whole plant by releasing many of them from the rings to the other leaves of the same plant. After 6 weeks, size measurements of eggs, adult females, and adult males were made using a microscopic micrometer. Twenty of each kind were measured and statistically analyzed.

RESULTS

Initial Screening and Evaluation of Plants

Plant evaluation and the scoring procedure were based on the degree of damage caused by the two-spotted spider mite infestation. Although inoculation was made to all leaves except the 4 at the top, the lower leaves suffered earlier and sustained more damage than did upper leaves. As the mite population developed, the damage symptoms moved upward to include even the apical growth points of the plants in many lines.

The first symptoms were yellow or rusty specks along the midrib and main veins of the leaf, ultimately spreading over the whole leaf. Next, entire leaves became yellow and necrotic spots appeared. On susceptible plants, leaves would become almost entirely necrotic and covered with webbing. Such leaves would soon abscise. On highly susceptible plants, the terminal growth became infested and entire plants died.

The tested lines responded differently in regard to the amount of damage caused by the mites. Analyses of variance (Tables 2 and 3) show significant differences among screened plants in both spring and fall tests. The lines, 'Hot Pants', 'Rainbow Star', 'Stoplight', 474-2', and 'Pink Satin', were highly susceptible in both tests, while '7729-9', '7729-8', and 'Chariot' had the highest resistance (Table 4). All

Table 2. Analysis of variance of means of scores of *Impatiens* lines screened during Spring 1979

Source of variation	Degrees of freedom	Mean squares	F-value
Lines	26	3.26	10.55**
Error	54	0.31	
Total	80		

**Means are significantly different at 0.01 level.

Table 3. Analysis of variance of means of scores of *Impatiens* lines screened during Fall 1979

Source of variation	Degrees of freedom	Mean squares	F-value
Lines	26	4.02	19.16**
Error	54	0.21	
Total	80		

**Means are significantly different at 0.01 level.

Table 4. Mean scores for degree of susceptibility for lines screened in spring and fall and for the amount of cuticle on leaves of the same plants[a]

Line	Spring test scores	Fall test scores	Amount of cuticle mg/disk
Hot Pants	5.0 a	5.0 a	0.067 k
Rainbow Star	5.0 a	5.0 a	0.074 hij
Stoplight	4.7 ab	4.7 ab	0.082 cdefg
474-2	4.7 ab	5.0 a	0.082 cdefg
Pink Satin	4.7 ab	4.7 ab	0.079 fghij
625-1	4.3 abc	5.0 a	0.075 ghij
Purple Silk	4.3 abc	4.0 bcd	0.080 defgh
Red Coat	4.3 abc	4.3 abc	0.082 cdefg
Red Magic	4.0 abcd	4.3 abc	0.084 bcde
Summer Star	4.0 abcd	4.3 abc	0.071 jk
October Charm	3.7 bcde	4.0 bcd	0.076 fghij
452-1	3.7 bcde	3.7 cde	0.086 bcde
7	3.3 cdef	3.0 ef	0.072 ijk
12	3.3 cdef	3.3 de	0.082 cdefg
473-1	3.3 cdef	3.3 de	0.087 bcd
Star Fire	3.3 cdef	3.7 cde	0.088 abc
16	3.0 defg	3.0 ef	0.083 bcde
21	3.0 defg	3.0 ef	0.084 bcde
78236	3.0 defg	3.0 ef	0.085 bcde
Ring Master	2.7 efgh	3.0 ef	0.086 bcde
14	2.3 fghi	2.3 fg	0.080 defgh
622-1	2.3 fghi	2.0 g	0.084 bcde
7884-C	2.3 fghi	2.3 fg	0.085 bcde
77109-1D	2.3 fghi	2.3 fg	0.084 bcde
Chariot	2.0 ghi	1.7 gh	0.094 a
7729-8	1.7 hi	1.7 gh	0.089 abc
7729-9	1.3 i	1.0 h	0.090 ab

[a]Means with the same letter are not significantly different at the 0.05 level as tested by Duncan's multiple range test.

other lines exhibited degrees of resistance somewhere between these two groups.

It was noted that the variation among lines was minimal during the first 3 weeks of infestation, but it developed rapidly afterwards. This would seem to be related to the time required for the mite population to build up. The number of mites on leaves was not counted, but the relative amount of damage to the leaves is generally considered to be a good indication of the size of a mite population. It was noted also that some lines have the capability of regenerating new growing points at the lower nodes, but in most cases rapid attack takes place even before completion of new leaf expansion. The first death of an entire plant occurred in the end of the seventh week of the infestation when 1 replicate of 'Hot Pants' died. The other 2 replicates of this line and all 3 replicates of the line 'Rainbow Star' died in the end of the eighth week. At this time, 2 replicates of the line '7729-9' and 1 replicate of the line '7729-8' showed no mite damage. The other replicates of these 2 lines were little affected. The same situation with these 4 lines happened again in the fall when the screen was duplicated, except that all replicates of '7729-9' were undamaged. The relative resistance of the other lines also was similar for both tests.

Leaf cuticle

This test was conducted to measure the degree of association of resistance to the two-spotted spider mite in the screened *Impatiens* plants with the amount of leaf cuticle. The tested lines differed significantly in their cuticle content (Table 5).

Table 5. Analysis of variance of means of amount of cuticle (mg/disk) of *Impatiens* lines

Source of variation	Degrees of freedom	Mean squares	F-value
Lines	26	0.000114	7.94**
Error	54	0.000014	
Total	80		

**Means are significantly different at 0.01 level.

The lowest amount was found to be in 'Hot Pants' and the highest in the line 'Chariot' (Table 4). Also, higher amounts could be noted in the lines '7729-9' and '7729-8'. The correlation coefficient (r) between cuticle thickness and degree of susceptibility was -0.66. Due to Snedecor and Cochran (1980), this value is significantly high, since absolute tabulated r value equals 0.487 at 1% and 25 degrees of freedom.

Crosses and Selfs for Progeny Evaluation

The 16 lines selected for selfing and hybridization were: '7', '12', '14', '16', '21', 'Hot Pants', '452-1', '474-2', '622-1', '625-1', '7729-8', '7729-9', '7884C', '77109-1D', 'Red Coat', and 'Ring Master'. These lines were of different rating scores including the extremes. Pollination attempts were tried nearly every day during September, October, and November 1979 with little success compared to the great number of flowers involved. Crosses using some lines, such as '7884C' and '625-1', were unsuccessful whether the lines were used as a male or female parent. The number of seeds produced from successful crosses ranged from 2 to 53 with 1 to 7 seeds per seed pod. Selfing gave a higher number of seeds with totals of 3 to 18 seeds and 3 to 21 seeds per pod.

Hybrids with seed counts lower than 8 were discarded as were those hybrids that did not have a successful reciprocal. This left 9 pairs of reciprocal crosses for testing. Likewise, only 7 of the selfed lines gave 8 or more seeds and were used in subsequent tests.

Feeding preference

For hybrids   Hybrids from reciprocal crosses showed no significant differences in food preference by the two-spotted spider mites (Table 6). This means that all hybrid

Table 6. Average number of mites per disk of hybrid groups from reciprocal crosses

Reciprocal crosses female x male	Hybrid groups I	Hybrid groups II	Total	t-value[a]
7729-9 x Ring Master	5.00	4.25	9.25	0.00
Ring Master x 7729-9	4.75	4.50	9.25	
7729-9 x 452-1	4.00	6.00	10.00	0.00
452-1 x 7729-9	5.50	4.50	10.00	
7729-9 x 77109-1D	3.50	5.00	8.50	-0.707
77109-1D x 7729-9	5.75	4.25	10.00	
7729-9 x Hot Pants	3.75	5.75	9.50	-0.485
Hot Pants x 7729-9	5.00	5.50	10.50	
7729-9 x 7729-8	4.00	3.75	7.75	-2.828
7729-8 x 7729-9	4.50	4.25	8.75	
7729-9 x 7729-1D	5.00	4.50	9.50	-0.707
77109-1D x 7729-8	4.75	5.25	10.25	
77109-1D x 452-1	4.25	5.50	9.75	-0.343
452-1 x 77109-1D	5.50	4.75	10.25	
77109-1D x Hot Pants	4.75	5.25	10.00	0.00
Hot Pants x 77109-1D	5.00	5.00	10.00	
452-1 x 474-2	5.25	4.25	9.50	-1.00
474-2 x 452-1	5.25	5.25	10.50	

[a]None of these values are significant.

groups do not differ from each other in acceptance as a food source for the starved mites. For this reason, only one clone from each hybrid was maintained for the progeny evaluation for damage.

For selfed progenies An evaluation of the feeding preference of mites among the progenies of selfs showed that significant differences for this factor existed in progenies of the lines '7', 'Hot Pants', '452-1', '7729-8', '7729-9', and 'Ring Master' (Tables 7, 8, 9, 10, 11, and 12, respectively), while the selfed progeny of the line '21' showed no significant difference (Table 13). Only 3 plants from each of the progenies that showed variation in feeding preference were kept for further testing and the others were discarded.

Table 7. Analysis of variance of preference for food by the two-spotted spider mite of line '7' selfed progeny

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	19	1.079	2.54**
Error	60	0.425	
Total	79		

**Means are significantly different at 0.01 level.

Table 8. Analysis of variance of preference for food by the two-spotted spider mite for 'Hot Pants' selfed progeny

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	19	1.816	4.64**
Error	60	0.391	
Total	79		

**Means are significantly different at 0.01 level.

Table 9. Analysis of variance of preference for food by the two-spotted spider mite for progeny of selfed '452-1' line

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	19	1.289	2.46**
Error	60	0.525	
Total	79		

**Means are significantly different at 0.01 level.

Table 10. Analysis of variance of preference for food by the two-spotted spider mite for progeny of selfed '7729-8' line

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	19	4.226	9.57**
Error	60	0.442	
Total	79		

**Means are significantly different at 0.01 level.

Table 11. Analysis of variance of preference for food by the two-spotted spider mite for progeny of selfed '7729-9' line

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	19	3.392	4.68**
Error	60	0.725	
Total	79		

**Means are significantly different at 0.01 level.

Table 12. Analysis of variance of food preference by the two-spotted spider mite for progeny of selfed 'Ring Master' line

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	7	2.071	4.32**
Error	24	0.479	
Total	31		

**Means are significantly different at 0.01 level.

Table 13. Analysis of variance of food preference by the two-spotted spider mite for progeny of selfed '21' line

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	15	1.367	1.75
Error	48	0.781	
Total	63		

Evaluation for damage This test was performed only once in Spring 1980 to test the differences in resistance to mites of 16 selected hybrids and inbreds. These plants showed significant differences in resistance to the pest (Table 14). The lines '452-1x474-2', 'Hot Pants ⓧ', '77109-1DxHot Pants', '21 ⓧ', and '452-1 ⓧ' were the least resistant, while the lines, '7729-9 ⓧ', '7729-9 x 7729-8', '7729-9 x Ring Master', and '7729-8 ⓧ' had the highest resistance (Table 15). All other lines were in between.

Table 14. Analysis of variance of scores of the selected progeny plants

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	15	3.976	19.09**
Error	32	0.208	
Total	47		

**Means are significantly different at 0.01 level.

Table 15. Score means of the selected hybrid and selfed plants of the test run in 1980[a]

The Plant	
452-1 x 474-2	5.0 a
Hot Pants ⊗	4.7 ab
77109-1D x Hot Pants	4.7 ab
21 ⊗	4.3 abc
452-1 ⊗	4.3 abc
Ring Master ⊗	4.0 bcd
77109-1D x 452-1	3.7 cde
7729-8 x 77109-1D	3.3 def
7729-9 x Hot Pants	3.3 def
7 ⊗	3.0 ef
7729-9 x 77109-1D	3.0 ef
7729-9 x 452-1	2.7 fg
7729-8 ⊗	2.0 gh
7729-9 x Ring Master	2.0 gh
7729-9 x 7729-8	1.7 h
7729-9 ⊗	1.3 h

[a]Means followed by the same letters are not significantly different at the 0.05 level as tested by Duncan's multiple range test.

Tests on Selected Parent and Progeny Plants

Seven lines having different degrees of resistance to the two-spotted spider mites were selected from the parents. An additional 10 hybrids between some of these parents or in one case a self of a parent with different resistance levels were selected also. The results of the tests performed with these lines follow.

Preference for oviposition

On leaf disks   The highest number of eggs per day (mean 5.9/female) was oviposited on '474-2' disks, and the least (mean 0.1/female) on '7729-9' (Table 16). Analysis of variance of mean eggs deposited on disks of different tested lines showed that significant differences existed among these lines (Table 17).

In Tanglefoot rings on attached leaves   This technique showed higher means of oviposition than the disks technique. The highest number of eggs per day (mean 11.8/female), and the least (mean 0.1/female) were in rings on the lines '747-2' and '7729-9', respectively, the same as with the previous technique (Table 16). Significant differences among lines were found in this test also (Table 18).

Table 16. Means of daily rate of oviposition on disks and rings, average life cycle of female spider mite in rings and on whole leaves, food preference, and leaf pH[a]

Plant	Daily oviposition per female per		Life cycle in days		Food preference	Leaf pH
	Disk	Ring	In rings for females	On whole leaves for females		
474-2	5.9 a	11.8 a	12.3 g	12.0 d	5.6 bc	6.546 bcd
77109-1D x 452-1	4.7 b	7.9 b	12.3 g	12.0 d	7.3 a	6.352 def
7729-9 x Hot Pants	4.6 b	7.9 b	16.0 cd	13.0 c	1.3 f	6.226 efg
452-1 x 474-2	2.6 c	6.8 bc	13.6 e	15.6 b	5.0 cd	6.903 a
77109-1D x Hot Pants	2.2 d	4.8 de	16.0 cd	15.6 b	1.0 fg	6.376 cdef
7729-9 x 77109-1D	2.0 de	4.1 ef	12.6 fg	12.6 cd	3.0 e	6.400 cdef
Hot Pants	1.8 ef	5.7 cd	13.6 e	12.3 cd	2.6 e	6.500 bcde
77109-1D	1.5 fg	5.7 cd	12.3 g	13.0 c	1.3 f	6.783 ab
7729-9 x 452-1	1.5 fg	5.6 cd	16.3 c	15.6 b	4.6 d	6.640 abc
7729-8 x 77109-1D	1.4 fg	3.2 f	12.6 fg	12.6 cd	6.3 b	6.213 fg
7729-9 x Ring Master	1.3 gh	3.4 f	15.3 d	15.3 b	0.3 g	6.863 a
7729-9 x	1.0 hi	0.9 g	18.3 a	18.0 a	1.3 f	6.563 bcd
452-1	0.7 ij	3.2 f	16.0 cd	15.0 b	2.3 e	5.996 g
7729-9 x 7729-8	0.6 ij	0.9 g	13.3 ef	13.0 c	2.6 e	6.210 fg
Ring Master	0.5 j	0.8 g	16.0 cd	15.3 b	3.0 e	6.786 ab
7729-8	0.4 jk	0.3 g	17.3 b	17.3 a	2.3 e	6.650 abc
7729-9	0.1 k	0.1 g	18.3 a	17.6 a	0.6 fg	6.690 ab
Mean	1.9	4.3	14.8	14.4	3.0	6.511

[a]Means with the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 17. Analysis of variance of daily rate of oviposition per adult female using leaf disks technique

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	8.184	199.18**
Error	34	0.041	
Total	50		

**Means are significantly different at 0.01 level.

Table 18. Analysis of variance of daily rate of oviposition per adult female using tanglefoot ring technique

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	31.042	60.98**
Error	34	0.501	
Total	50		

**Means are significantly different at 0.01 level.

Life cycle response of mites

Rings technique Significant differences in the length of life cycle of female mites on different *Impatiens* lines were found (Table 19). The longest life cycle seems to be on the resistant lines and the shortest on the susceptible lines (Table 16). The range between the extreme lines was from 12.3 days to 18.3 days, with average of 14.8 days for the population.

Table 19. Analysis of variance of average life cycle of the two-spotted spider mite on plants using tanglefoot ring technique

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	13.460	52.81**
Error	34	0.255	
Total	50		

**Means are significantly different at 0.01 level.

Using the whole leaf This procedure also showed significant differences in length of life cycle of the female two-spotted spider mite on different lines (Table 20). The shortest one (12.0 days) was on the highly susceptible lines and longest (18.0 days) was on the highly resistant lines with the average life cycle in the population being 14.4

Table 20. Analysis of variance of average life cycle of spider mites on plants using whole leaf technique

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	12.505	63.77**
Error	34	0.196	
Total	50		

**Means are significantly different at 0.01 level.

days (Table 16).

Feeding preference

This experiment was performed to test the nonpreference mechanism of resistance. The test showed significant differences among the 17 tested lines in feeding preference by the two-spotted spider mite (Table 21). Generally, the resistant lines showed a lower degree of preference by the starved mites than did the susceptible lines (Table 16). The least preferred line was '7729-9', and the most preferred was '474-2'. The line '7729-9' when crossed with 'Ring Master' resulted in a less preferred hybrid, while the same line when crossed with the resistant line '7729-8' resulted in hybrid of higher preference than either parent. Similar results were found when the highly susceptible line '452-2' was crossed with the line '77109-1D'. The other hybrids showed intermediate situations.

Table 21. Analysis of variance of average mite number per detached leaf as a measure of food preference

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	13.083	51.33**
Error	34	0.255	
Total	50		

**Means are significantly different at 0.01 level.

pH preference test

This experiment was performed primarily to correlate the leaf pH of the 17 lines with food preference. Three pH measurements, at different times, were taken from each line. The analysis of variance of pH means showed that significant differences existed among the lines (Table 22). The range of the pH value of the lines was between 5.996 and 6.903 (Table 16). A correlation test between the pH of the lines and their preference as food by the two-spotted spider mite gave a correlation coefficient (r) value of -0.16, indicating a very low degree of association between these 2 characters.

Size tests of eggs, adult females, and adult males

The tests showed significant differences of sizes of eggs, females, and males of the two-spotted spider mites (Tables 23, 24, and 25). The highly susceptible line,

Table 22. Analysis of variance of average leaf pH of the 17 *Impatiens* plants

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	0.203	8.71**
Error	34	0.023	
Total	50		

**Means are significantly different at 0.01 level.

Table 23. Analysis of variance of egg size of the two-spotted spider mite on the 17 lines of *Impatiens* (parents and progeny)

Source of variation	Degrees of freedom	Mean squares	F-value
Plants	16	0.00091	5.92**
Error	323	0.00015	
Total	339		

**Means are significantly different at 0.01 level.

'474-2', showed the largest egg, female, and male size, while the highly resistant line, '7729-9' had the smallest eggs, females, and males (Table 26). The mean egg size ranged from 0.125 to 0.150 mm, mean female size ranged from 0.397 to 0.553 mm, and mean male size ranged from 0.260 to 0.348 mm.

Table 24. Analysis of variance of female size of the two-spotted spider mite on 17 lines of *Impatiens* (parents and progeny)

Plants	16	0.0259	24.62**
Error	323	0.0010	
Total	339		

**Means are significantly different at 0.01 level.

Table 25. Analysis of variance of male size of the two-spotted spider mite on 17 lines of *Impatiens* (parents and progeny)

Plants	16	0.01466	63.33**
Error	323	0.00023	
Total	339		

**Means are significantly different at 0.01 level.

Table 26. Means of egg size, adult female size, and adult male size of the two-spotted spider mite on some *Impatiens* lines and some of their progenies[a]

Plant	Egg size (mm)	Female size (mm)	Male size (mm)
474-2	0.150 a	0.553 a	0.352 a
7729-8	0.141 b	0.448 cde	0.275 fg
7729-9 x 7729-8	0.141 b	0.418 g	0.293 cde
77109-1D x 452-1	0.138 bc	0.397 h	0.348 a
77109-1D x Hot Pants	0.138 bc	0.463 bcd	0.267 ghi
Ring Master	0.136 bcd	0.461 bcd	0.267 ghi
7729-9 x 452-1	0.136 bcd	0.477 b	0.295 cd
7729-8 x 77109-1D	0.136 bcd	0.482 b	0.290 cde
7729-9 x 77109-1D	0.133 bcde	0.467 bc	0.275 fg
7729-9 x Ring Master	0.131 cde	0.427 efg	0.267 ghi
Hot Pants	0.130 cde	0.447 cdef	0.283 ef
452-1	0.130 cde	0.482 b	0.310 b
77109-1D	0.128 de	0.428 efg	0.288 de
7729-9 x Hot Pants	0.128 de	0.425 efg	0.300 c
7729-9 ⊗	0.128 de	0.428 efg	0.260 i
7729-9	0.125 e	0.442 defg	0.263 hi
452-1 x 474-2	0.125 e	0.423 fg	0.273 fgh

[a]Means with the same letter are not significantly different according to Duncan's multiple range test.

DISCUSSION AND CONCLUSION

The screening of the 27 *Impatiens* lines indicated a wide variation among these lines to the infestation by the two-spotted spider mite in both the spring and fall tests. However, the response of each line was very similar in the two tests which means that the resistance is true resistance and not due to the pest avoidance or escape from infestation (Painter, 1951; Russell, 1978).

The variation in resistance among the lines is reflected, not only by the amount of damage caused by mites to the plants, but also by the speed at which the damage occurred. For example, the leaves of 'Hot Pants' started abscising six weeks after infestation, and 1 plant of this line dies at the end of the seventh week. On the other hand, the leaves of '7729-9' did not start to abscise until after 10 weeks. None of the 3 replicates of this line had died after 2 years from the initial infestation, even with constant exposure to spider mites. Although these plants always showed symptoms of mite damage, the damage was restricted to the older leaves and plant growth, although slower than normal, continued throughout this period.

On susceptible lines, damage symptoms first showed as yellow or rusty specks along the midribs and the main veins, eventually spreading to the entire leaf. The leaf turns rusty in color, then necrosis occurs and webs are formed. With resistant lines, similar damage symptoms occur, but they start near the edge of the leaf and then spread inward. The time required to cover the whole leaf is much longer than that required for the susceptible lines. In all tested lines, attacks by the two-spotted spider mites start on the lower leaves of the plant, and as the infestation develops, the population moves toward the top. The death of plants of the susceptible lines occurs after the growing points of these plants are attacked by the mites. The appearance of webbing in the growing point area is generally a signal that the mite population is sufficiently large to cause the death of a plant. Slow increase in population size of the mites on resistant plants would seem to be associated with the fact that they never reach the growing point in sufficient numbers to interfere with the new growth of the plant. Thus, the resistant plant will continue to put out new leaves even though a mite infestation is present on the lower leaves of the plant. The slower growth of an infested resistant plant as compared to an uninfested one could be related to the loss of photosynthetic capacity by the plant as a result of the mite damage to the older leaves.

One possible reason for the differences in mite susceptibility among the lines could be the thickness of cuticle of the leaves, since a thick cuticle is believed to be one of the ways that a plant might repel a tetranychid mite population (Jesiotr et al., 1979). The thickness of cuticle was not estimated but indicated by the measured amount of cuticle on the leaf surfaces, since the thickness of cuticle is proportional to the amount of cuticle. The constant value in this relationship would be 1/area of disks x the density of cuticle.

A high negative correlation between the amount of cuticle on the leaves and the amount of infestation indicates that the cuticle is at least one of the factors that protect the plants from the attack of the two-spotted spider mite. This agrees with Jesiotr et al.'s (1979) statement. Because the differences in the resistance to the two-spotted spider mites among different *Impatiens* lines can be attributed in part to the differences in the amount of cuticle, the inheritance of cuticle characteristics should be tested along with the other mechanisms which also may be important in influencing the level of resistance.

Tests for resistance to spider mites, using progenies of reciprocal crosses between resistant and susceptible lines was used to detect whether the resistance is being controlled by genes in cytoplasm or in the nucleus. Dunn (1974) found that inheritance of resistance to root aphid in lettuce plants is controlled by both cytoplasmic and genetic factors. No other examples regarding cytoplasmic inheritance to resistance against animal pests were found in the literature, but many can be found for other organisms.

The lack of significant differences in the amount of mite damage between the 2 hybrids in any of the pairs of reciprocal crosses would indicate a definite absence of any cytoplasmic influence among these plants in the control of resistance to the two-spotted spider mite. Even the reciprocals from the cross between '7729-9' and 'Hot Pants', showed no variability regarding feeding preference by the mites. In contrast, considerable variability was found for this trait among the selfed progenies.

From the results of evaluating progenies of the selected 16 hybrid and self plants, it is clear that the resistant lines, '7729-9' and '7729-8', when selfed, maintained the resistance in their progenies, and when crossed with susceptible lines, such as 'Hot Pants' and '452-1', produced intermediate progenies. This could lead to a conclusion that the resistance in the *Impatiens* plants is polygenic and partially dominant which is similar to what was found in strawberry plants by Chaplin et al. (1968). Also, selfing the resistant line, 'Ring Master', showed loss in resistance which might be attributed to nonadditive gene action and/or a loss of vigor which is similar to what was found in strawberry plants.

Although a feeding preference test is not a means of final judgment as to the presence or absence of resistance in a plant, it should indicate the possibility that resistance is present in the nonpreferred plant. Other tests should be performed, however, to substantiate the fact that a particular plant is resistant (Painter, 1951).

Damage evaluation of the progenies reflects the degree of resistance in plants, but not the mechanism(s) responsible for expressing such resistance. It is a means used to simplify and detect the resistance in the tested plant without exploring the details and complexity of resistance.

The number and type of mechanisms involved in resistance to a pest are believed to affect the stability of resistance and are of as much importance as the number of genes that control these mechanisms (Russell, 1978). Therefore, many tests concerning this belief were conducted with 17 selected parents, selfs, and hybrids of Impatiens plants.

Regarding oviposition response, both techniques, the leaf disk and the tanglefoot rings, showed that the highly susceptible line '474-2' was the most preferred for oviposition, and that the highly resistant lines '7729-9', '7729-8', and 'Ring Master' were the least preferred for oviposition. The complexity of the genetic control of this character can be illustrated by the hybrids '77109-1D x 452-1' and '7729-9 x Hot Pants'. In these hybrids, the crossing of highly resistant lines with medium resistant lines resulted in individuals that were quite susceptible. The fact that only 1 individual was tested from hybrids between heterozygous parents precludes any interpretation from these data concerning the genetic control of this character. It should be noted, however, that in all parental combinations except one, the hybrids received more eggs than did either parent. This would point out the need for testing all individuals of a hybrid population in order to identify the ones with the highest levels of resistance.

Overall, these 2 techniques for measuring oviposition produced quite similar results, particularly among the 4 most susceptible and the 4 most resistant lines. The variation in relative oviposition among the intermediate lines could be related to the suggestion by Van de Vrie et al. (1972) that oviposition depends upon the nutrition status, and using leaf disks may tend to affect this factor. Therefore, relying on leaf disk technique for the actual rate of oviposition of mites on *Impatiens* lines is not recommended. Although the same mean number of eggs was found on the resistant line '7729-9' in both techniques, twice as many were found on '474-2', the susceptible line, using the ring technique as compared to the leaf disk method. Since the 2 techniques gave fairly similar results on the resistant end of the scale, however, it should be possible to use the disk technique as a preliminary screen to eliminate most of the susceptible individuals. The ring method then could be used for the final evaluation because it is considered to be closer to natural conditions and thus a more accurate evaluation.

The life cycle tests with the ring and the whole leaf techniques showed averages of 14.8 and 14.5 days, respectively, indicating that being forced to live on the top surface of the leaf as in the ring technique had no effect on the development of the mites when given the environmental conditions provided for these tests. With very few exceptions, the data from these tests were very similar, indicating that either method would be satisfactory for obtaining this kind of information. The life cycle results also agreed with the oviposition tests in regard to the most susceptible and the most resistant lines. There was a considerable variation in the results from the 2 kinds of tests for lines that were between the extremes though. The hybrid '7729-9 x 7729-8' had a low oviposition rate but provided for a relatively short life cycle. If '7729-9' were crossed with '452-1', however, the length of the hybrid's life cycle was closer to that of the most resistant line than would be expected from its relative position among the lines in regard to oviposition. The line '77109-1D' and most hybrids with this line have a much shorter life cycle than would be expected, not only when compared to the oviposition data, but also when one takes into account the results of the original screens. This would indicate the possible presence in the _Impatiens_ plant of at least 2 factors that influence the growth of the two-spotted spider mite. The major one, of course, would be the influence on general growth of the mites and the other possibility would be a factor that influences the length of the life cycle. A highly resistant line, such as '7729-1D', would have factors for both restricted mite growth and long life cycle, while '77109-1D' would provide for a short life cycle along with restricted mite growth and have a lower level of overall mite resistance than '7729-9'. This idea is substantiated by the fact that the sizes of the eggs and females of '77109-1D' were quite similar to those of the resistant lines.

The relationship between mite size and the level of plant resistance to mites should be explained by the postulation of Painter (1951) that the feeding of insects during developmental stages on resistant plants results in individuals that are smaller in size through the indirect effect of antibiosis.

Tests for the size of eggs, females, and males of the two-spotted spider mite were done after the mite population was allowed to develop for 6 weeks. According to the average life cycle on the Impatiens plants, 3 generations were developed during that period. In 3 generations, the resistant plants should have affected the size of the individuals and maybe their eggs.

A very weak relationship exists between the size of eggs and mites and the other factors being measured. In regard to egg size, the largest were found on '474-2' as to be expected, but the smallest were on the hybrid between '474-2' and '452-1'. The mites also produced small eggs on '7729-9' but produced larger eggs on the sister line '7729-8' and the hybrid between the two. These unexpected results for egg size might not be representative of the effect of antibiosis on eggs, since some of them might have come from adults of the first or second generation that were still alive and laying eggs.

Measurements of the length of adult males and females did not estimate the level of resistance as compared to the original ratings any better than did egg size. The problem of measuring mites from the early generations as a result of a random sampling could be enhanced by the large variation among the lines in the length of the life cycle. This possible confusion among generations could be reduced by periodically moving the mites to another plant of the same line, but this would make this technique even more time consuming.

A question would have to be raised as to the value of this technique, considering the amount of time needed and the questionable accuracy of the results, when compared to some of the other methods of measuring mite resistance.

The food preference test is one of the most common tests run by plant breeders to measure resistance levels to a pest. This technique adds a new dimension to the testing because it allows the mites to have a preference for their food source rather than being restricted to a certain plant. For this reason, it was not surprising that the data obtained from this test were quite different in regard to food preference, and the hybrid of '7729-8' and '77109-1D' was next to the highest as the most preferred food source. In contrast, 'Hot Pants', which was rated as highly susceptible on the mite damage tests, had a relatively low preference score, and its hybrid with '77109-1D' was among the lines that were least preferred. Although nonpreference for food by itself generally is not considered to be a satisfactory measure of total plant resistance, perhaps it should be considered as one of the factors needed in a highly resistant plant. Further study of this characteristic could be beneficial.

Leaf pH was measured to determine if it could be a factor in the preference for a food source by the mites. A low correlation between these 2 factors, however, negated the possibility of any real relationship. Neither did leaf pH seem to be related to the results of any of the other tests used in this study to evaluate the level of mite resistance. This would agree with the results obtained by Bart J. Fiori[1].

In addition to being used to obtain information concerning possible interactions between the mites and the host

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[1]Bart J. Fiori, NE Regional Plant Introduction Station, NYS Agr. Exp. Sta., Geneva, N.Y. 14456, personal communication.

plants, the various techniques also were evaluated as to whether they would be just as accurate but more efficient than the regular screening procedure of inoculating entire plants with mites and reading the symptoms after 6 to 8 weeks. The major problem in using whole plants is the amount of space required to screen a large number of hybrid populations. A plant breeder must incorporate all the desired horticultural characteristics into a new cultivar along with the mite resistance. Thus, he must look at a large number of plants to achieve this goal. The use of leaf disks in the oviposition test or the single leaf in the food preference would be very efficient in regard to space requirements and the amount of time required. Both these tests identified the most highly resistant line with the oviposition results being the most similar to those of the whole plant screens. The food preference test could be very important though because it involves the original choice by the mite as to the plant on which it wishes to feed. Because these 2 tests are efficient, it should be relatively easy to use both on the hybrid populations, and then screen only those plants that were highly resistant in both tests with the whole plant damage evaluation. The life cycle and the leaf cuticle measurement tests also would be of value, but would be in the later stages of the breeding program when all the parents had high levels of resistance.

When attempting to relate the lines used in this study to the original plant introduction (P.I.) lines from which they originated, it could be found that the highly susceptible and highly resistant lines had many common ancestors. The only parent common to the resistant lines that was not in the parentage of the susceptible lines was P.I. 354253. Since this line is closely related to 2 other P.I. lines that were in the parentage of both the susceptible and the resistant lines, its importance as a source of resistance could be questioned. A fact that would seem to be of more importance is the appearance of P.I. 354259 in the pedigrees of all the highly susceptible lines. It is possible that genes from this line control a susceptibility trait and are dominant to, or in some other way, inhibited the action of, the genes for resistance found in the other lines. The impossibility of developing homozygous inbreds from these Impatiens lines makes a regular genetic study of the resistance characteristic difficult. On the basis of the information obtained in this study, however, it would seem that a plant breeder could eventually produce cultivars with a high level of resistance to the two-spotted spider mite by continual testing and the elimination of all the susceptible plants from his breeding program as soon as possible.

LITERATURE CITED

Bailey, J. C., R. E. Furr, B. W. Hanny, and W. R. Meredith, Jr. 1978. Field populations of twospotted spider mites on sixteen cotton genotypes at Stoneville, MS, 1977. J. Econ. Entomol. 71: 911-912.

Beck, S. D. 1965. Resistance of plants to insects. Annual Rev. Entomol. 10: 207-232.

Boudreaux, H. B. 1958. The effect of relative humidity on egg-laying, hatching, and survival in various spider mites. J. Insect. Physiol. 2: 65-72.

Boudreaux, H. B., and G. Dosse. 1963. Concerning the names of some common spider mites. In J. A. Naegele (ed.) Adv. Acarol. 1: 350-364.

Briggs, J. B., and F. H. Alston. 1969. Sources of pest resistance in apple cultivars. Report of East Malling Res. Stn. 1968: 159.

Cagle, L. R. 1949. Life history of the two-spotted spider mite. Virginia Agric. Exp. Stn. Tech. Bull. 113.

Chaplin, C. E., L. P. Stoltz, and J. G. Rodriguez. 1968. The inheritance of resistance to the two-spotted mite, Tetranychus urticae Koch in strawberries. Proc. Am. Soc. Hort. Sci. 92: 376-380.

Chaplin, C. E., L. P. Stoltz, and J. G. Rodriguez. 1970. Breeding behavior of mite resistant strawberries. J. Am. Soc. Hortic. Sci. 95: 330-333.

DaCosta, C. P., and C. M. Jones. 1971. Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in Cucumis sativus L. Science 172: 1145-1146.

Dahms, R. G. 1972. The role of host plant resistance in integrated insect control. p. 152-167. In M. G. Jotwani and W. R. Young (eds.) Control of sorghum shoot fly. Oxford and IBH Pub. Co., New Delhi, India.

Dunn, J. A. 1974. Study on inheritance of resistance to root aphid, Pemphigus bursarius, in lettuce. Ann. Appl. Biol. 76: 9-18.

Fraenkel, G. 1959. The raison d'etre of secondary plant substances. Science 129: 1466-1470.

Gerson, U. 1979. Silk production in Tetranyus (Acari: Tetranychidae). Recent Advances in Acarology 1: 177-188.

Gilbert, J. C., J. J. Chinn, and J. S. Tanka. 1966. Spider mite tolerance in multiple disease resistance commercial type tomatoes. Proc. Am. Soc. Hortic. Sci. 89: 559-562.

Harrison, R. A., and A. G. Smith. 1961. The influence of temperature and relative humidity on the development of eggs and on the effectiveness of ovicides against Tetranychus telarius L. (Ararina: Tetranychidae). New Zealand J. Sci. 4: 540-549.

Hazan, A., U. Gerson, and A. S. Tahori. 1974. Spider mite webbing. L. The production of webbing under various environmental conditions. Acarologia 16: 68-84.

Hazan, A., U. Gerson, and A. S. Tahori. 1975. Quantitative evaluation of the feeding of the carmine spider mite Tetranychus cinnabarinus (Boisd.) (Acari: Tetranychidae). Entomol. Res. Bull. 65: 515-521.

Henneberry, T. J. 1962. The effect of plant nutrition on the fecundity of two strains of two-spotted spider mite. J. Econ. Entomol. 55: 134-137.

Herne, D. H., J. E. Cranham, and M. A. Easterbrook. 1979. New acaricides to control resistant mites. Recent Advances in Acarology 1: 95-104.

Holloway, P. F., and E. A. Baker. 1968. Isolation of plant cuticles with zinc chloride-hydrochloric acid solution. Plant Physiol. 43: 1878-1879.

Huffaker, C. B., M. van de Vrie, and J. A. McMurtry. 1969. The ecology of tetranychid mites and their natural control. Annual Rev. Entomol. 14: 125-174.

Hussey, N. W., and W. J. Parr. 1963. Dispersal of the glasshouse red spider mite, Tetranychus urticae Koch (Acarina, Tetranychidae). Entomol. Exp. Appl. 6: 207-214.

Hussey, N. W., W. J. Parr, and C. D. Crocker. 1957. Effect of temperature on the development of Tetranychus telarius L. Nature 179: 739-740.

Jeppson, L. R., H. H. Keifer, and E. W. Baker. 1975. Mites injurious to economic plants. University of California Press, Berkeley, California. 614 pp.

Jesiotr, L. J., Z. W. Suski, and T. Badowska-Czubik. 1979. Food quality influences on a spider mite population. Recent Advances in Acarology 1: 189-196.

Johnson, B. 1956. The influence of aphids on the glanular hairs of tomato plants. Plant Pathol. 5: 130-132.

Kono, T., and C. S. Papp. 1977. Handbook of agricultural pests. State of California Dept. of Food and Agric., Sacramento, California.

Kooistra, E. 1971. Red spider mite tolerance in cucumber. Euphytica 20: 47-50.

LaMotte, C. E., D. J. Nevins, and C. R. Stewart. 1973. Plant Physiology Experiments Book. Iowa State University, Ames, Iowa.

Markkula, M., K. Roukka, and K. Trittanen. 1969. Reproduction of _Myzus persicae_ (Sulz.) and _Tetranychus telarius_ (L) on different _Chrysanthemum_ cultivars. _Ann. Agric._ Fenn. 8: 175-183.

McEnroe, W. D., and K. Dronka. 1966. Color vision in the adult female two-spotted spider mite. Science 154: 782-784.

McEnroe, W. D., and K. Dronka. 1969. Eye of the female two-spotted spider mite, _Tetranychus urticae_. II. Behavioral analysis of the photoreceptors. Ann. Entomol. Soc. Am. 62: 466-499.

McKinny, K. B. 1938. Physical characteristics on the foliage of beans and tomatoes that tend to control some small insect pests. J. Econ. Entomol. 31: 630-631.

Nelson, R. D., and E. M. Stafford. 1972. Effect of gamma radiation on the biology and population suppression of the two-spotted spider mite, _Tetranychus urticae_ Koch. Hilgardia 41: 299-432.

Owens, J. C. 1975. An explanation of terms used in insect resistance to plants. Iowa State J. Res. 49: 513-517.

Painter, R. H. 1951. Insect resistance in crop plants. The University Press of Kansan, Manhattan, Kansas.

Patterson, C. G., R. Thurston, and J. G. Rodriguez. 1974. Two-spotted spider mite resistance in Nicotiana species. J. Econ. Entomol. 76: 341-343.

Patterson, C. G., D. E. Knavel, T. R. Kemp, and J. G. Rodriguez. 1975. Chemical basis for resistance to Tetranychus urticae Koch. in tomatoes. Environ. Entomol. 4: 670-674.

Ponti, O.M.B. De. 1977. Resistance in Cucumis sativus L. to Tetranychus urticae Koch. 2. Designing a reliable laboratory test for resistance based on aspects of the host-parasite relationship. Euphytica 26: 641-654.

Ponti, O.M.B. De. 1979. Resistance in Cucumis sativus L. to Tetranychus urticae Koch. 5. Raising the resistance level by the exploitation of transgression. Euphytica 28: 569-577.

Ponti, O.M.B. De, and H. Inggamer. 1976. Technical note: An improved leaf disk technique for biotests. Euphytica 25: 129-130.

Regev, S., and W. W. Cone. 1975. Evidence of farnesol as a male sex attractant of the two-spotted spider mite, Tetranychus urticae Koch. (Acarina: Tetramychidae). Environ. Entomol. 4: 307-311.

Regev, S., and W. W. Cone. 1980. The monoterpene citronellol as a male sex attractant of the two-spotted spider mite, Tetranychus urticae Koch. (Acarina: Tetranychidae). Environ. Entomol. 9: 50-52.

Rodriguez, J. G. 1951. Mineral nutrition of the two-spotted spider mite, Tetranychus bimaculatus Harvey. Ann. Entomol. Soc. Am. 44: 511-526.

Russell, G. E. 1978. Studies in the agricultural and food sciences. Plant breeding for disease resistance. Butterworth Publ. Inc., London.

Saito, Y. 1977. Study on the spinning behavior of the spider mite (Acarina: Tetranychidae). 1. Method for the quantitative evaluation in the mite webbing, and the relationship between webbing and walking. Jpn. J. Appl. Entomol. Zool. 21: 27-34.

Sances, F. V., J. A. Wyman, and I. P. Ting. 1979. Morphological response of strawberry leaves to infestation of two-spotted spider mite. J. Econ. Entomol. 72: 710-713.

Schalk, J. M., A. K. Stoner, R. E. Webb, and H. F. Winters. 1975. Resistance in eggplant, Solanum melongena L., and nontuber-bearing Solanum sp. to carmine spider mite. J. Am. Soc. Hortic. Sci. 100: 479-481.

Schuster, M. F., and F. G. Maxwell. 1976. Resistance to two-spotted spider mite in cotton. Mississippi Agric. For. Exp. Stn. Bull. 821.

Schuster, M. F., F. G. Maxwell, J. N. Jenkins, and W. L. Parrott. 1972. Mass screening seedlings of Gossypium sp. for resistance to the two-spotted spider mite. J. Econ. Entomol. 65: 1104-1107.

Smith, F. F., and E. W. Baker. 1968. Names of the two-spotted spider mite and the carmine spider mite to be redesignated. U.S. Dept. Agric. Coop. Econ. Ins. Rep. 18(47): 1080.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods. The Iowa State University Press. pp. 508.

Snetsinger, R., C. P. Balderston, and R. Craig. 1966. Resistance to the two-spotted spider mite in Pelargonium. J. Econ. Entomol. 59: 76-78.

Soans, A. B., D. Pimentel, and J. S. Soans. 1973. Resistance in cucumber to the two-spotted spider mite. J. Econ. Entomol. 66: 380-382.

Stoner, A. K. 1970. Selecting tomatoes resistant to spider mites. J. Am. Soc. Hortic. Sci. 95: 78-80.

Stoner, A. K., and T. Stringfellow. 1967. Resistance of tomato varieties to spider mites. Proc. Am. Soc. Hortic. Sci. 90: 324-329.

Stoner, A. K., J. A. Frank, and G. A. Gentile. 1968. The relationship of glandular hairs on tomatoes to spider mite resistance. Proc. Am. Soc. Hortic. Sci. 93: 532-538.

Suski, Z. W., and J. A. Naegele. 1963. Light responses in the two-spotted spider mite. 1. Analysis of behavioral response. Adv. Acarol. 1: 435-445.

Suski, Z. W., T. Badowska, and M. Olszak. 1975. The influence of fertilizers and soil management systems on the population of Tetranychid mites in the apple orchards. Fruit Science Reports 2(1): 85-95.

Thorsteinson, A. J. 1960. Host selection in phytophagous insects. Ann. Rev. Entomol. 5: 193-218.

van de Vrie, M., J. A. McMurtry, and C. B. Huffaker. 1972. III. Biology, ecology, and pest status, and host-plant relations of Tetranychids. Hilgardia 41: 343-432.

Wharton, G. W. 1963. Equilibrium humidity. Adv. Acarol. 1: 201-208.

Winston, P. W. 1963. Humidity relations in the clover mite, Bryobia praetiosa Koch. Ecology 44: 669-678.

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